Identification of Neuron-Specific Enolase in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagents:

1X Automation Buffer

3% Hydrogen Peroxide

Antibody Diluent

Citrate Buffer

DAB Chromagen

Hematoxylin

Antibody Information

Blocking Serum: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #008-000-001

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary antibody: Mouse anti-Neuron Specific Enolase
Lab Vision
Fremont, CA 94539
www.labvision.com
1-800-828-1628
Catalog#MS-1717-P1

Negative control serum: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Secondary antibody: Biotinylated Mouse anti-Horse IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #BA-2001

Label antibody: Vector Standard Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

Staining Procedure

-Positive Control Tissue: Pancreas: Islets of Langerhans
-Stain Localization: Cytoplasm / Nucleus

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking Techniques using the decloaker.
Add 500 ml D/W to the pan of the decloaker.
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.
Decloak for 5 minutes. Pressure_____
Depressurize for 10 minutes.
Remove pan top and cool for 10 min.Temp_____
Rinse in D/W, 2x for 3 min each

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block with 10% Normal Horse Serum for 20 minutes.
Lot# _____Reconstituted Date_____

6. Apply Avidin/Biotin block Lot#_____ New Kit yes / no
Apply avidin block - 15 min @ RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min @ RT.
Wipe excess block

DO NOT RINSE SECTIONS WITH BUFFER.

7. Apply primary antibody (NSE) at 1:800 and incubate for one hour at room temperature.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (NSE) and use this to make a 1:800 dilution. Apply and incubate for one hour at room temperature.
Lot#_____ Reconstituted Date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply Horse anti-mouse at 1:500 for 30 minutes.
Lot#_____ Reconstituted Date_____

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Vector Elite label and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)
Exp. Date _____ New kit yes / no

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp. Date_____ New kit yes no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

18. Coverslip
updated 8/18/04